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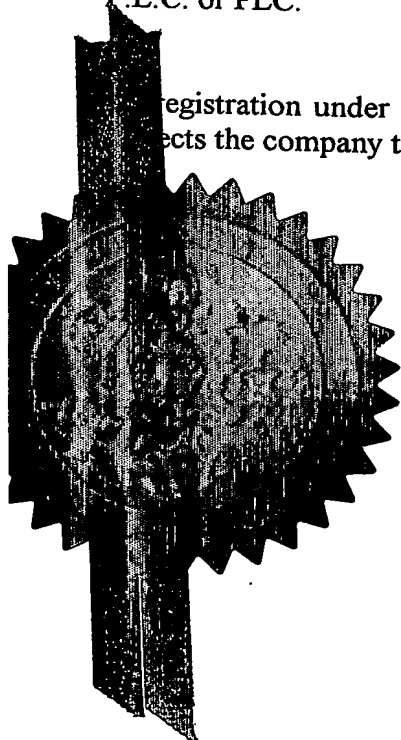
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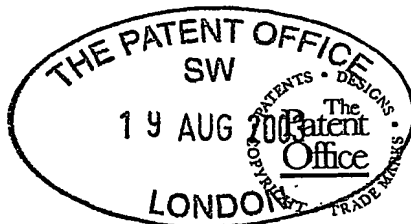


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Denmark

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

Denmark

05660873004

4. Title of the invention

PROCESS

5. Name of your agent (if you have one)

D Young & Co

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21 New Fetter Lane  
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David Alcock

023 8071 9500

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## PROCESS

The present invention relates to a process for the production of a food product and to a  
5 food product produced by the process.

## BACKGROUND ART

Certain protein-containing food products, such as acidified dairy products like drinking  
10 yoghurt and milk juice drinks, require a stabiliser to stabilise the protein system against  
aggregation, sedimentation and separation. The major protein present in cows' milk is  
casein, which constitutes about 80% of the total protein content. The remaining protein  
in cows' milk is termed "whey protein" and consists predominantly of beta-lactoglobulin  
and alpha-lactalbumin. Cows' milk is comprised of water and milk solids. The milk solids  
15 include fat and milk solid non-fat (MSNF) which is made up of protein together with  
lactose and various minerals.

High-ester (HE) pectin has traditionally been used as a stabiliser in protein-containing  
food products such as acidified protein beverages. Pectin is a structural polysaccharide  
20 found in fruit and vegetables and may, for example, be extracted from citrus peel. At a  
molecular level, pectin consists of a linear chain of galacturonic acid units linked through  
 $\alpha$ -1,4 glycosidic bonds (the 'smooth region'). This regular structure is interrupted by  
rhamnopyranosyl residues with side chains of neutral sugars (the 'hairy region'). Pectin  
molecules have a molecular weight of up to about 200,000 and a degree of  
25 polymerisation of up to about 800 units. A proportion of the carboxylic acid groups of the  
galacturonic acid units are methyl esterified. The properties of pectin depend on the  
degree of esterification, which is less than 50% for low-ester (LE) pectin and more than  
50% for high-ester pectin.

30 HE pectin is known to have the ability either to prevent aggregation of casein micelles or  
to be the cause of it, depending on the pH of the system. The micellar casein-HE-pectin  
system switches from hydrocolloid non-adsorption and depletion flocculation at neutral  
pH 6.7 to hydrocolloid adsorption and polymeric stabilisation at pH 4 [2, 4]. Therefore,  
although HE pectin is an effective stabiliser at acidic pH, at neutral pH conditions it is  
35 incompatible with the milk proteins and separates the milk into two phases.

Depletion flocculation of casein micelles involves exclusion of the polymer pectin chains from the space between the colloidal casein micelles, which induces an attractive interaction between the casein micelles. If the depletion attraction is strong enough, segregative phase separation occurs resulting in two immiscible aqueous phases, where the upper phase is rich in pectin and poor in casein micelles, while the lower phase is, on the contrary, mainly loaded with casein micelles [1, 2, 4]. At a low pectin concentration, the phase volume occupied by the pectin molecules is low. At increasing pectin concentrations, the occupied volume and the osmotic pressure of the pectin solution increase, which induces a stronger flocculation of the casein micelles. Finally, at a certain pectin concentration, the phase separation takes place. About 0.20% HE pectin is needed to induce phase separation in skimmed milk at pH 6.7 [2].

Pectin is a non-adsorbing polymer when it is in solution with skimmed milk at pH 6.7, but when lowering the pH to 5.3, the pectin molecule adsorbs onto the casein micelle. If the pectin concentration is low and insufficient for full coverage of the casein micelles, bridging flocculation occurs. When increasing the pectin concentration further, the casein micelles become fully coated and the system re-stabilises. Thereby, the attraction between the casein particles is lowered and stable conditions are obtained [2]. Although the adsorption of pectin onto the casein micelles is possible at pH conditions above the isoelectric point of caseins ( $pI \sim 4.6$ ), the pH of efficient stabilisation is generally restricted to about pH 3.5 to 4.4 where the pectin and casein carry sufficient opposite net charges for effective adsorption [4].

This mechanism is used to stabilise acidic protein beverages against protein aggregation. Efficient polymeric stabilisation is achieved by the combination of high molecular weight, high surface coverage and a blockwise distribution of galacturonic acid groups. Therefore in theory, the best polymeric stabiliser would be a copolymer with a strongly adsorbing terminal with low solvent affinity and a voluminous dangling end with high solvent affinity to increase repulsion upon forced polymer overlap [4]. For stabilisation of acidic protein beverages, HE pectin has generally been considered to be the hydrocolloid of choice. Although HE pectin has a lower charge density than low-ester (LE) pectin and thereby a weaker electrostatic interaction with casein micelles, it generally serves as a more effective stabiliser of casein dispersions. It is believed that a smaller region of the HE pectin molecule interacts with the casein particle, allowing a

more substantial part of the pectin dangling chain to be freed from solvent interaction thus preventing protein aggregation through steric hindrance [7].

The difference in the stabilisation characteristics of HE pectin at different pH values determines the applications in which HE pectin may be used as a stabiliser and the stage in the production process when the HE pectin may be added.

The acidification of protein beverages can be achieved by the addition of an acid (for example an acidic fruit juice). Acidification can also be achieved via fermentation. However, for acidified protein beverages containing HE pectin, these two processes are technically distinct from each other:

For directly acidified protein beverages like milk juice drinks, addition of juice and/or acid directly to milk results in the formation of acid casein particles of uncontrollable size.

These particles are typically too big to be kept in suspension resulting in a non-stable acidic protein beverage with a sandy mouth-feel upon heat treatment. In the production of directly acidified protein beverages, the destabilising effect of high molecular weight HE pectin at neutral pH is used to advantage. The HE pectin is typically added to the milk before acidification and, under the neutral pH conditions, induces separation of the milk into two phases. The molecular exclusion effect of pectin concentrates the intact casein micelles in a lower, protein rich phase and leaves the pectin-rich whey phase virtually free of micelles. The casein phase has the properties of a liquid and can be dispersed into the whey phase in the form of droplets by stirring. The more shear applied to the system, the smaller the drops become and the more like an oil-in-water emulsion the system becomes. The subsequent rapid pH drop through direct acidification freezes the casein droplets in their native form at the size they had in the neutral milk and thereby creates acid casein particles of controlled size [5]. During the acidification process the caseins become reactive to each other but are prevented from aggregation and precipitation due to the presence of HE pectin that forms the above-mentioned protective coat around the casein micelles [13].

Thus, for directly acidified protein beverages, HE pectin is added at neutral pH and induces phase separation. Strong mechanical stirring is then used to keep the precipitated casein proteins in suspension. The system is rapidly acidified freezing the casein proteins in suspension. The casein proteins are stabilised by the high-ester

pectin molecules under the acidic conditions and are thereby prevented from sedimentation in the final application.

For fermented milk products, HE pectin cannot be used in the same way. Production of fermented milk products typically involves the steps of pasteurisation of the milk base, followed by inoculation with bacteria and finally fermentation. During fermentation by bacteria, the pH of the milk is reduced gradually and slowly in contrast to the rapid pH drop in the above application. Thereby, a disintegration of the casein micelles takes place that thickens or gels the milk into yoghurt [5, 13].

Addition of traditional, high molecular weight HE pectin to the milk before fermentation would induce phase separation as described above, when applied in concentrations required for efficient protein stabilisation of the final fermented drink. Phase separation in this application would be undesirable because the characteristic yoghurt structure and its subsequent texture impact would be lost. Furthermore the precipitated casein micelles cannot be kept in suspension by stirring in this application. Mechanical stress and incorporation of oxygen is normally avoided during fermentation of milk to give the live bacteria the best fermentation conditions. Therefore, strong mechanical stirring to keep the separated casein micelles in suspension cannot be applied. Moreover, the pH drops too slowly to freeze the casein structures. In summary, high molecular weight HE pectin is not typically effective if added to milk before fermentation and is instead added after fermentation to protect the acidified proteins against aggregation [14].

For fermented dairy products which contain live culture the final product is not typically pasteurised or sterilised. It is therefore of utmost importance to pasteurise the milk prior to fermentation, to avoid contamination during fermentation and contamination of the final product. When HE pectin is applied to fermented milk drinks containing live culture, it must be sterilised as well to avoid contamination of the product. As discussed above, known commercial HE pectin products cannot be added to the milk prior to pasteurisation, inoculation and fermentation and therefore the pectin needs to be sterilised separately. This typically involves the heat sterilisation of aqueous pectin solutions that require additional processing and equipment to both dissolve and heat the pectin. The pectin is typically in the form of a pectin syrup which is sterilised by heating and subsequently added to the already fermented milk base. The additional pectin sterilisation process requires additional tank capacity and heat equipment and increases

the energy costs. The alternative and much simpler method of adding pectin directly to the fermented milk in the form of a dry mix with sugar is not applicable due to the contamination risk.

- 5 For manufacturers of fermented milk products it would be easier and cheaper (e.g. in terms of process equipment and energy requirement) to operate with a stabiliser which can be added to the milk prior to fermentation i.e. before the slow acidification. Before fermentation, it is common to pasteurise the milk in order to avoid contamination but also, which is of significant importance, to heat denature the whey proteins to get optimal
- 10 yoghurt structure. This process would be greatly simplified if the pasteurisation of milk could be combined with the pasteurisation of the stabiliser. The stabiliser would then not have to be sterilised separately. Additionally, the method of addition of the stabiliser would be more flexible, since both direct addition as dry mix with sugar and dispersion in a saturated sugar solution could be used as alternatives to the dissolved stabiliser
- 15 solution.

- It is desirable to seek a stabiliser of fermented protein food products that is compatible with proteins in the food material such as milk and which can be added to the food material, resist a pasteurisation together with the food material, prevent flocculation and
- 20 phase separation during fermentation and finally stabilise the acidified proteins after fermentation and optionally after a final pasteurisation to prolong the shelf-life.

- One of the difficulties in providing a stabiliser that may be added prior to pasteurisation, inoculation and fermentation is incompatibility of the stabiliser with the proteins (e.g. milk
- 25 proteins) at neutral pH. Generally, proteins (e.g. milk proteins) and polysaccharides (e.g. HE pectin) are incompatible at a sufficiently high bulk concentration and under conditions inhibiting formation of inter-biopolymer complexes. This mainly occurs at a sufficiently high ionic strength (exceeding 0.2), pH values above the protein isoelectric point and at a total biopolymer concentration above 3-4% [1, 12, 16], whereas alkaline
- 30 pH conditions and low ionic strengths enhance the co-solubility [1, 4]. Furthermore, protein-polysaccharide incompatibility usually increases on heating and with protein denaturation [6, 9, 12, 15]. Therefore, the important pasteurisation of milk, in order to denature the whey proteins before fermentation, would be likely to enhance incompatibility even further in a blend of casein micelles and HE pectin at neutral pH
- 35 conditions.



The conditions for a limited compatibility are different for systems including neutral (e.g. locust bean gum and guar gum), sulphated (e.g. carrageenan) or carboxyl-containing (e.g. pectin) polysaccharides and the compatibility typically decreases in the order  
 5 sulphated>neutral>carboxyl-containing polysaccharides [6, 7, 12]. The effect of several hydrocolloids on the stabilisation of casein micelles has been tested with locust bean gum and guar gum of the neutral polysaccharides; gum arabic, CMC (carboxymethylcellulose), pectin, hyaluronic acid and alginates of the carboxyl-containing polysaccharides; and agarose, heparin, chondroitin sulphates, cellulose sulphate,  
 10 fucoidan and carrageenan of the sulphated polysaccharides. Only carrageenan induced significant stabilisation at pH 6.8 [11].

High molecular weight and rigidity of macromolecule chains tend to increase incompatibility and normally, linear polysaccharides are more incompatible with proteins  
 15 than branched polysaccharides. In general, the larger the difference in molecular weight and in hydrophilicity, the more pronounced the incompatibility of the biopolymers [12]. The following examples are found in literature:

- A system of HE pectin and skimmed milk at natural pH clearly demonstrates depletion flocculation [1, 4, 8]. The destabilisation and subsequent phase separation  
 20 is even known as a tool to efficiently concentrate proteins from skimmed milk on a technological scale [10]. Depletion flocculation of casein micelles at neutral pH occurs whatever the type of pectin used (low-ester, low-ester amidated and high-ester pectin). The phase separation boundary is obtained at lower polysaccharide concentrations with LE pectin than for HE pectin [16].
- Mixing guar gum (neutral polysaccharide) with skimmed milk at neutral pH leads to  
 25 phase separation, but the phase boundary shifts to higher guar concentrations, when the molecular weight of guar gum is reduced through degradation [17]. Locust bean gum, guar gum and hydrolysed guar gum with reduced molecular weight (all neutral polysaccharides) behave differently in a micellar casein system at neutral pH. Since  
 30 locust bean gum and hydrolysed guar gum have a lower intrinsic viscosity than the initial guar gum sample, they occupy a smaller volume in the medium per molecule than the guar gum chains. The exclusion of the polymer thus occurs to a lesser extent, resulting in a decreased aggregation of casein micelles at the same polysaccharide concentration [18].

- At pH 7, CMC readily precipitates casein from both skimmed milk and from casein model solutions. Less CMC is required when higher viscosity types are used, i.e. types with higher molecular weight [4].

5 At present, the only well-known and readily available commercial product on the market for fermented protein beverage applications which can be added prior to fermentation is soluble soybean polysaccharide (SSPS), produced by Fuji Oil [19]. SSPS is a water-soluble polysaccharide extracted and refined from soybean. Fuji Oil Co., Ltd., Japan, has marketed SSPS under the brand name SOYAFIBE-S since 1993. SSPS is mainly  
10 composed of the dietary fibre of soybean and has relatively low viscosity and high stability in aqueous solution.

SSPS is a much more branched polymer than HE pectin with a rather short backbone and many more long side chains. HE pectin has a long backbone and just a few short  
15 side chains. The component sugars in SSPS are mainly galactose, arabinose, galacturonic acid but also include many others such as rhamnose, fucose, xylose and glucose. Gel filtration chromatographic analysis by HPLC shows that SSPS consist roughly of three components having approximate molecular weights of 550,000; 25,000 and 5,000. The major component of SSPS consists of long-chain rhamnogalacturonan  
20 and short-chain homogalacturonan, while citrus pectin consists of short-chain rhamnogalacturonan and long-chain homogalacturonan. For SSPS, homogenous galactosyl and arabinosyl neutral sugar side chains combine with the rhamnogalacturonan region through rhamnose and are longer than the galacturonosyl main backbone.

25 SSPS has a galacturonic acid content of about 20% [19] whereas pectin has a galacturonic acid content of at least 65%. The anion group of this acid probably binds to the surface of cationic protein particles so that SSPS protects the casein micelles. It is assumed that the adsorbed layer of SSPS is thick, because each molecule is rich in side  
30 chains of galactose and arabinose [19]. SSPS is soluble in both cold and hot water without gelation and shows a relatively low viscosity compared to the viscosity of other gums/stabilisers. Acid, heat or salts (e.g. Ca-salts) do not significantly affect the viscosity of SSPS in solution. Under acidic conditions, SSPS prevents protein particles from aggregation and precipitation.

Unlike HE pectin, the point of interest with SSPS is its ability to stabilise protein particles at low pH conditions without raising the viscosity of the acidified protein beverage. SSPS is reported to perform even if applied at an early stage of processing before fermentation, which allows the manufacturing process to be improved. SSPS shows good stabilising effect in lower pH products (below pH4.0). However, SSPS is less effective than HE pectin at higher pH such as around pH4.4 and/or high milk solid non-fat (MSNF) contents.

The need exists to provide alternative stabilisers which may be added during the production of fermented protein beverages prior to fermentation and preferably prior to the initial pasteurisation.

The present invention alleviates the problems of the prior art.

## **STATEMENT OF INVENTION**

In one aspect the present invention provides a process for the production of a food product comprising the steps of (i) contacting a food material with a stabiliser to provide a food intermediate; and (ii) fermenting the food intermediate; wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

In one aspect, the present invention provides a process for the production of a food product comprising the step of dissolving a stabiliser directly in a food material wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

In another aspect, the present invention provides a food product obtained or obtainable by the process of the present invention.

In a further aspect, the present invention provides use of a stabiliser for improving the texture and/or viscosity of a food product, wherein the stabiliser comprises a depolymerised pectin.

The term "food product" as used herein means a substance that is suitable for human or animal consumption. It will be readily understood that whilst the food product is the

product of the process as herein described, it may undergo further processing prior to consumption.

The term "stabiliser" as used herein means a substance which is capable of stabilising protein in a system with which it is contacted – so as to prevent or substantially reduce aggregation and/or sedimentation and/or separation. The "system" may, for example, be a food material comprising a protein, a food intermediate comprising a protein or a food product comprising a protein. Preferably the "system" is a food product comprising a protein.

The term "food material" as used herein means one or more ingredients of the food product.

The term "fermenting" as used herein typically means a process in which desirable chemical changes are brought about in an organic substrate through the action of microbes and/or microbial enzymes. The fermenting conditions typically include attaining and maintaining a specified temperature for a specified period of time. It will be readily appreciated that the temperature and duration may be selected in order to enable the biochemical processes associated with fermentation, especially the breakdown of organic compounds by micro-organisms to progress to a desired extent. The organic compounds may, for example, be carbohydrates, especially sugars such as lactose.

The term "depolymerised pectin" as used herein means a substance obtained or obtainable from naturally-occurring pectin by breaking it down into two or more fragments. Pectin has a backbone comprising repeated structural units and typically has a degree of polymerisation of up to 800 units. These repeated structural units are principally galacturonic acid residues and rhamnopyranosyl residues. The depolymerised pectin has chains of no greater than 250 units, such as chains of 15 to 250 units. Typically these units are galacturonic acid units. The naturally-occurring pectin may be broken down by any suitable depolymerisation method, such as various mechanical, chemical, thermal, enzymatic or irradiative methods or combinations of the same. Suitable depolymerisation methods include those discussed in Studies on Pectin Degradation, W. H. Van Deventer-Schriemer and W. Pilnik, Acta Alimentaria, vol. 16 (2), pp. 143-153 (1987). The term "depolymerised pectin" also includes those substances,

for example naturally-occurring substances, which have short chains of 15 to 250 units and in particular short galacturonan chains of 15 to 250 galacturonic acid units.

### **Advantages**

5

We have surprisingly found that a stabiliser comprising a depolymerised pectin can be applied directly to a protein-containing food material, such as milk, prior to fermentation and yet stabilise the resultant food product which may, for example, be a fermented dairy product.

10

Prior art stabilisers such as high molecular weight HE pectin induce phase separation if added to protein-containing food materials such as milk prior to fermentation. Therefore traditionally it has been necessary to add a stabiliser after fermentation in order to achieve the desired stabilisation of the food product.

15

A further advantage is that the method of addition of the stabiliser is more flexible, since both direct addition as dry mix with sugar and dispersion in a saturated sugar solution may be used as alternatives to the dissolved stabiliser solution.

20

We have also surprisingly found that a stabiliser comprising a depolymerised pectin dissolves more easily directly in a food material such as milk than other stabilisers such as HE pectin. The present stabiliser may therefore be dissolved directly in the food material avoiding the need for a separate dissolution step. This further simplifies the production process.

25

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

30

### **PREFERRED EMBODIMENTS**

#### **PROCESS**

As previously mentioned, in one aspect the present invention provides a process for the  
35 production of a food product comprising the steps of (i) contacting a food material with a

stabiliser to provide a food intermediate; and (ii) fermenting the food intermediate; wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

5 In one aspect, the present invention provides a process further comprising, before step (ii), the step of (i)(a) pasteurising the food intermediate. In other words, the present invention provides a process for the production of a food product comprising, in the following order, the steps of (i) contacting a food material with a stabiliser to provide a food intermediate; (i)(a) pasteurising the food intermediate; and (ii) fermenting the food  
10 intermediate; wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

The term "pasteurising" as used herein means attaining and maintaining a specified temperature for a specified period of time. The specified temperature is usually attained  
15 by heating. It will be readily appreciated that the temperature and duration may be selected in order to kill or inactivate certain bacteria, such as harmful bacteria. A rapid cooling step may follow.

We have surprisingly found that a stabiliser comprising a depolymerised pectin can be  
20 applied directly to a protein-containing food material, such as milk, prior to pasteurisation and fermentation and yet stabilise the resultant food product which may, for example, be a fermented dairy product.

This embodiment of the present invention is particularly advantageous when the food  
25 product does not undergo a final pasteurisation step, for example because it comprises a live culture. In applications such as these, this process allows the manufacturer of the food product to avoid separate pasteurisation of the stabiliser since the stabiliser and the food material may be pasteurised together prior to fermentation. This leads to benefits in terms of lower energy and equipment costs, reduced processing time and a simplified  
30 processing procedure. In particular the energy costs, tank capacity and heat equipment associated with the separate pasteurisation of the stabiliser are not required.

In one aspect, the present invention provides a process further comprising, before step (ii), the step of (i)(b) inoculating the food intermediate.

The term "inoculating" as used herein means introducing a micro-organism into a system. The micro-organism may, for example, be a bacterium and may be used to start a culture.

- 5 According to this aspect, the present invention may provide a process for the production of a food product comprising, in the following order, the steps of (i) contacting a food material with a stabiliser to provide a food intermediate; (i)(b) inoculating the food intermediate; and (ii) fermenting the food intermediate; wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

10

In a highly preferred aspect, the present invention provides a process for the production of a food product comprising, in the following order, the steps of (i) contacting a food material with a stabiliser to provide a food intermediate; (i)(a) pasteurising the food intermediate; (i)(b) inoculating the food intermediate; and (ii) fermenting the food

15

intermediate.

In a preferred aspect, the process further comprises the step of (iii) pasteurising the product of step (ii).

- 20 In another preferred aspect, the process further comprises the step of (iv) adding juice and/or acid to the product of step (i)(b) and/or to the product of step (ii) and/or to the product of step (iii).

### **Stabiliser**

25

As previously mentioned, the stabiliser for use in the present invention comprises a depolymerised pectin.

- 30 Preferably, the depolymerised pectin has a viscosity at 25°C in a 1% solution of from greater than 1mPa·s to about 5mPa·s, such as from about 2mPa·s to about 5mPa·s, more preferably from about 2mPa·s to about 4mPa·s, more preferably about 3mPa·s. Typically the viscosity is measurable in accordance with the method described below.

- 35 In a preferred aspect, the depolymerised pectin has a viscosity at 25°C in a 5% solution of 15 to 400 mPa·s, such as 20 to 300 mPa·s, 20 to 200 mPa·s, 20 to 100 mPa·s or 25 to

50 mPa·s. Typically the viscosity is measurable in accordance with the method described below.

5 In one preferred aspect, the stabiliser has a viscosity at 25°C in a 5% solution of greater than 150mPa·s, such as greater than 150mPa·s to 400mPa·s, for example greater than 150mPa·s to 300mPa·s or greater than 150mPa·s to 200mPa·s. Typically the viscosity is measurable in accordance with the method described below.

10 Preferably the depolymerised pectin is an essentially linear carbohydrate polymer. This is in direct contrast to SSPS which is an essentially branched carbohydrate polymer.

15 The term "carbohydrate polymer" as used herein means a molecule comprising substantially only carbon, hydrogen and oxygen atoms and which comprises repeated structural units. Preferably at least 95% of the atoms in the carbohydrate polymer are carbon, hydrogen or oxygen atoms, more preferably at least 98%, such as 99% or 100%.

The carbohydrate polymer may comprise a main backbone substituted with one or more side chains.

20 The term "essentially linear" means that the total number of atoms in the backbone is greater than the total number of atoms in the side chains.

25 As previously mentioned, the depolymerised pectin comprises no greater than 250 repeated structural units. Preferably the depolymerised pectin comprises 15 to 250 units, such as 15 to 200 units, 20 to 150 units or 30 to 100 units. Preferably the repeated structural units are galacturonic acid residues and/or rhamnopyranosyl residues.

30 In one aspect, the depolymerised pectin comprises no greater than 250 galacturonic acid units. Preferably the depolymerised pectin comprises 15 to 250 galacturonic acid units, such as 15 to 200 galacturonic acid units, 20 to 150 galacturonic acid units, or 30 to 100 galacturonic acid units.

35 In a preferred aspect, the depolymerised pectin has a galacturonic acid content of at least 65%, such as at least 70% or at least 75% or at least 80%. The galacturonic acid content may be measured using the method described in [3].



In one aspect, preferably the depolymerised pectin has a degree of esterification of at least 50%, such as at least 60%, or at least 65%. In this aspect, preferably the depolymerised pectin has a degree of esterification from 50 to 90% such as from 50 to 85%, more preferably from 65 to 75%. In a highly preferred embodiment the depolymerised pectin has a degree of esterification of about 70%.

A depolymerised pectin having a degree of esterification at least 50% may be particularly advantageous in a process for the production of a yoghurt, especially a yoghurt beverage, although for a yoghurt a degree of esterification below 50% may also be suitable.

In another aspect, preferably the depolymerised pectin has a degree of esterification of less than 50%, such as less than 40% or less than 30%.

The depolymerised pectin may be prepared from pectin by any suitable depolymerisation method and the pectin may be obtained from any suitable source. Examples of sources of pectin are citrus fruits (lemon, lime, orange, grapefruit, mandarine, tangerine, pommelo etc.) apple, sugarbeet root, carrot, sunflower head residue, onion, peach, grape berry, mango, guava, squash, pumpkin, tomato, apricot, banana, bean and potato. The pectin may be a commercially available pectin. In one aspect, the depolymerised pectin is obtainable, preferably obtained from citrus fruits.

Alternatively, the depolymerised pectin may be prepared from one of the sources of pectin directly, without first isolating the pectin, and the depolymerised pectin may subsequently be extracted. For example, the depolymerisation of pectin can be carried out in harvested plant material, after processing of plant material, for example in plant residues from juice production before or after drying. The depolymerisation can also be carried out during pectin processing: before the pectin extraction, during pectin extraction or in the pectin juice or concentrate after the pectin extraction. It is also possible to carry out the depolymerisation in wet precipitated pectin, during drying of pectin or in dry pectin after the pectin has been isolated.

Depolymerisation methods include various mechanical, chemical, thermal, enzymatic and irradiative methods or combinations of any thereof, in particular those methods

capable of breaking down long chains such as long galacturonan chains into shorter chains, for example into chains of 15 to 100 repeated structural units such as galacturonic acid units.

- 5 The chemical depolymerisation methods could be acid, alkaline, oxidative or reductive methods. Acid depolymerisation is shown in Mazoyer et al. UK Patent Application GB 2,311,024 (1997). Alkaline depolymerisation of pectin by  $\beta$ -elimination was studied by Renard et al., in Visser & Voragen, *Pectins and Pectinases* pp. 603-608 (1996) and Sajjaanantakul et al., *J. Food Sci.*, 54: 1272-1277 (1989). Oxidative depolymerisation of  
10 polysaccharides was studied by Miller in *Biochemical and Biophysical Research Communications* Vol 141, pp. 238-244 (1986). Examples of thermal depolymerisation studies are given in Merrill and Weeks, *J. Am. Chem. Soc.*, 67: 224 (1945), Mitchell et al. US Patent 5,498,702 (1996). Enzymatic depolymerisation of pectin by polygalacturonase, pectin lyase or pectate lyase has been widely recommended for  
15 depolymerisation of pectic substance both in plant material as well as in pectin extracts.

The depolymerised pectin may be prepared by the following general procedure. Pectin, for example a commercially available pectin, is dissolved in demineralised water at 85-90°C to constitute a 5% solution. The pH of the solution is adjusted to 5.5 by addition of  
20 20% sodium carbonate solution. The solution is kept at 80°C for 2 to 8 hours until the viscosity of the solution (measured at 25 °C) is lowered to 30 to 50 mPa.s. The pH is subsequently lowered to 3.5 by addition of 30% nitric acid and the mixture is cooled to room temperature. Pectin is precipitated out of the solution by pouring the mixture in 3 volume parts 80% isopropyl alcohol under good agitation. After approximately four hours  
25 the precipitate is separated from the liquid by filtration through a cloth and washed with another part of 80 % isopropyl alcohol. After pressing in the cloth the material is dried in a ventilated oven at 60°C during the night. Finally the dried product is milled to obtain depolymerised pectin.

- 30 The stabiliser comprising depolymerised pectin may be provided in any suitable form, in particular as a dry mix, as a solution or as a dispersion. As previously mentioned, step (i) of the process is contacting a food material with a stabiliser. This may be done in any suitable manner. In one aspect, the stabiliser is dry mixed with sugar and then dissolved in water to provide a stabiliser solution. The stabiliser solution is then mixed with a food  
35 material such as milk with stirring to provide the food intermediate.

In addition to the depolymerised pectin, the stabiliser may comprise other components such as dextrose. In one embodiment, the stabiliser comprises a depolymerised pectin and a high molecular weight high ester pectin.

5

The term "high molecular weight, high ester pectin" means a pectin having a viscosity in a 5% solution at 25°C of more than 400 cP and a degree of esterification of at least 50%.

In one embodiment the stabiliser comprises essentially only a depolymerised pectin.

10

### **Food material**

As previously mentioned the food material comprises a protein. Preferably the protein is of animal, and/or vegetable, and/or microbial origin. The protein may have been isolated from a suitable source, for example as a protein powder or protein isolate.

15

A suitable food material comprising protein of animal origin may be, for example, cows' milk, buffalo milk, goat milk or sheep milk. A suitable food material comprising protein of vegetable origin may be or may be derived from, for example soy, rice, wheat, oat, pea or coconut.

20

In a preferred aspect, the food material comprises protein of animal origin and protein of vegetable origin. Preferably, the food material comprises protein of animal origin. Preferably the protein is a milk protein.

25

In one preferred aspect the food material comprises milk. In one aspect the milk is selected from the list consisting of cows' milk, buffalo milk, goat milk and sheep milk. The milk may be whole fat milk or defatted milk. In one aspect the food material comprises milk and a protein of vegetable origin. The protein of vegetable origin could be, for example, soya protein or rice protein.

30

Preferably, the milk has a milk solid non-fat content of 0.1 to 25 wt%, preferably 3 to 25 wt%, more preferably 9 to 25 wt%.

The food material may comprise other food ingredients such as emulsifiers, hydrocolloids, preservatives, antioxidants, colourings and flavourings.

### **Pre-fermentation Pasteurisation**

5

As previously mentioned, in one aspect, the process of the present invention comprises the step of (i)(a) pasteurising the food intermediate.

10

Preferably the pasteurising step (i)(a) takes place at a temperature of at least 80°C, preferably at least 90°C. More preferably the pasteurising step (i)(a) takes place at a temperature of at least 95°C, such as 95°C to 100°C. In one aspect, preferably the pasteurising step (i)(a) takes place at a temperature of about 95°C. In one aspect, preferably the pasteurising step (i)(a) takes place at a temperature of at least 100°C.

15

Preferably the pasteurising step (i)(a) takes place over a period of 1 to 20 minutes, preferably 5 to 15 minutes, such as about 10 minutes.

In a preferred aspect the pasteurising step (i)(a) takes place at a temperature of about 95°C for about 10 minutes.

20

### **Inoculation**

As previously mentioned, in one aspect, the process of the present invention comprises the step of (i)(b) inoculating the food material.

25

30

Preferably the inoculation step (i)(b) comprises the addition of a live food-grade micro-organism. Preferably the live food-grade micro-organism is a live food-grade bacterium. Preferably the live food-grade bacterium is capable of influencing the taste and/or aroma and/or texture of the food product. In one aspect preferably the live food-grade bacterium is capable of influencing the taste of the food product. In another aspect preferably the live food-grade bacterium is capable of influencing the aroma of the food product. In a further aspect preferably the live food-grade bacterium is capable of influencing the texture of the food product. Preferably the live food-grade bacterium is capable of influencing the taste, aroma and texture of the food product.

35

The term "capable of influencing the taste and/or aroma and/or texture" means capable of altering the taste and/or aroma and/or texture of the food product as compared with the food product in the absence of the live food-grade bacterium.

- 5 Preferably the live food-grade micro-organism is a probiotic bacterium.

The term "probiotic bacterium" means a bacterium that has a beneficial effect on human and/or animal health. A probiotic bacterium may act in the gastrointestinal tract and/or in the urogenital tract. The health benefits of the probiotic bacterium may include:

10

- antagonistic effects on pathogenic bacteria
- beneficial metabolic activities such as production of vitamins or bile salt hydrolase activity
- stimulation of the immune response

15

- protection against early events in carcinogenesis
- improved recovery from intestinal disorders

In a preferred aspect, the live food grade micro-organism is selected from the list consisting of *Bifidobacteria*, *Streptococcus thermophilus*, *Lactobacilli* and mixtures thereof. Preferably the live food grade micro-organism is selected from the list consisting of *Bifidobacteria*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus* and mixtures thereof. In a preferred aspect, the live food-grade micro-organism comprises *Lactobacillus bulgaricus* and/or *Streptococcus thermophilus*, preferably *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

25

Preferably the live food-grade micro-organism is added in an amount of 0.01 to 0.05 wt% of the food intermediate. Preferably the live food-grade micro-organism is added in an amount of 0.01 to 0.03 wt%.

### 30 **Fermentation**

As previously mentioned, the process of the present invention comprises the step of (ii) fermenting the food intermediate.

Preferably the fermentation step (ii) takes place at a temperature of from 30 to 50°C, preferably 35 to 45°C, more preferably 37 to 43°C.

5 In a preferred aspect, the fermentation step (ii) takes place at a temperature of about 42°C.

Preferably the fermentation step (ii) takes place over a period of 2 to 48 hours.

10 In a preferred aspect, the fermentation step (ii) takes place at a temperature of about 42°C over a period of 2 to 10 hours, preferably 4 to 8 hours.

### **Post-fermentation Pasteurisation**

15 As previously mentioned, in one preferred aspect, the process of the present invention further comprises the step of (iii) pasteurising the product of step (ii).

20 Preferably the pasteurising step (iii) takes place at a temperature of at least 80°C, preferably at least 85°C. More preferably the pasteurising step (iii) takes place at a temperature of at least 90°C, such as 90°C to 100°C. In one aspect, preferably the pasteurising step (iii) takes place at a temperature of about 90°C. In another aspect, preferably the pasteurising step (iii) takes place at a temperature of above 100°C.

25 Preferably the pasteurising step (iii) takes place over a period of 5 to 30 seconds, preferably 10 to 20 seconds, more preferably about 15 seconds.

In a preferred aspect, the pasteurising step (iii) takes place at a temperature of about 90°C over a period of about 15 seconds.

30 This final post-fermentation pasturisation step may be included to provide a long shelf-life product. In a preferred aspect, the food product has a shelf-life of more than seven days, preferably more than 14 days, more preferably more than 28 days. In one preferred aspect the food product has a shelf-life of more than three months, preferably more than four months, preferably more than five months, such as more than six months.

### **pH Adjustment**

As previously mentioned, in another preferred aspect, the process further comprises the step of (iv) adding juice and/or acid to the product of step (i)(b) and/or to the product of  
 5 step (ii) and/or to the product of step (iii). Preferably the juice and/or acid is added to the product of step (ii) and/or to the product of step (iii). Preferably the juice and/or acid is added to the product of step (ii).

Preferably the juice is a fruit juice. Examples of suitable fruit juices include apple juice,  
 10 apricot juice, banana juice, grapefruit juice, grape juice, guava juice, lemon juice, lime juice, mandarine juice, mango juice, orange juice, peach juice, pommelo juice, pumpkin juice, squash juice, tangerine juice, tomato juice and mixtures thereof.

The juice may be a natural or a treated juice (such as a concentrated juice or a juice  
 15 having one or more components separated therefrom.) Preferably the juice is pasteurised at a temperature of at least 80°C, such as at least 85°C or at least 95°C prior to addition.

Preferably the acid is a food acid. Examples of suitable food acids include citric acid,  
 20 malic acid, and lactic acid. In this aspect, preferably the food acid is citric acid, lactic acid or a mixture thereof.

The addition of juice and/or acid may modify the pH of the system and typically lowers the pH of the system.  
 25

In a preferred aspect the pH of the food intermediate immediately prior to the fermentation step (ii) is, or is adjusted to pH 6.0 to 8.0, preferably pH 6.3 to 7.0, such as pH 6.5 to 7.0, more preferably about pH 6.7.

30 In a preferred aspect, the juice and/or acid is added to the product of the fermentation step (ii). Preferably, sufficient juice and/or acid is added to adjust the pH to less than pH 4.6, preferably less than pH 4.4, preferably less than pH 4.2, more preferably about pH 4.0.

## FOOD PRODUCT

In one aspect the present invention provides a food product obtained by the process of the present invention. In another aspect the present invention provides a food product  
 5 obtainable by the process of the present invention.

The food product obtainable, preferably obtained by the process of the present invention may be any suitable fermented protein-containing food product.

10 Examples of suitable food products include cheese, quarg, sour cream, imitation sour cream (e.g. with vegetable oil), dessert cream, fermented dessert products (such as set or stirred yoghurt desserts), frozen fermented products (such as frozen yoghurt or frozen, fermented ice cream), lassi drink, ayran, laban, buttermilk, kefir drink (lactic acid and alcohol fermentation), liquid yoghurt (such as drinking yoghurt), lactic acid bacteria  
 15 beverages, blends of fermented protein beverages and juice, pulp, fruit etc. based on e.g. milk, whey and/or soy (this could be yoghurt mixed with juice like a smoothie which is not the same as a milk juice drink directly acidified by the juice), fortified drinks (such as calcium-fortified drinking yoghurt) and protein enriched soft drinks. Other suitable food products include any of the above listed food products which comprise soy protein  
 20 in addition to or instead of milk protein.

In one aspect the food product is a beverage.

Preferably the food product is a fermented milk drink, preferably a yoghurt drink, more  
 25 preferably a drinking yoghurt drink.

The term "fermented milk drink" covers a food product produced by any kind of fermentation by any kind of organism.

30 The term "yoghurt drink" typically covers a milk product produced by fermentation by the combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The term yoghurt drink includes diluted milk drinks with a low MSNF content.

The term "drinking yoghurt drink" typically covers a milk product produced by  
 35 fermentation by the combination of *Lactobacillus bulgaricus* and *Streptococcus*



*thermophilus*. Drinking yoghurt drinks typically have a milk solid non-fat content of 8% or more. Furthermore, the live culture count for drinking yoghurt drinks is typically at least  $10^6$  cell forming units (CFU).

- 5 Preferably the food product contains a live food-grade micro-organism in an amount of from 0.01 to 0.05 wt%, more preferably 0.01 to 0.03 wt%, preferably, 0.02 wt%.

Preferably the food product contains the stabiliser in an amount of 0.1 to 5.0 wt%, preferably 0.2 to 4.0 wt%, preferably 0.3 to 3.0 wt%.

10

Preferably the food product contains the depolymerised pectin in an amount of 0.1 to 1.0 wt%, preferably 0.2 to 0.8 wt%, preferably 0.4 to 0.7 wt%. In one aspect preferably the food product contains the depolymerised pectin in an amount of no greater than 0.4 wt% such as 0.4 wt% to 0.1 wt%, or 0.4 wt% to 0.2 wt% or 0.4 wt% to 0.3 wt%.

15

Preferably the food product has a pH of less than pH 4.6, preferably less than pH 4.4, preferably less than pH 4.2, more preferably about pH 4.0 or less.

- 20 Preferably, the food product has a milk solid non-fat (MSNF) content of 0.1 to 20 wt%, preferably 1 to 15 wt%, more preferably 1 to 10 wt%. In one aspect, the MSNF content is less than 3 wt%. In a preferred aspect the MSNF content is at least 3 wt%. In a further preferred aspect, the MSNF content is at least 8 wt%.

- 25 Drinking yoghurts typically contain a minimum of 8% by weight of MSNF. Yoghurt drinks typically contain a minimum of 3% by weight of MSNF, whereas soft drinks, milk juice drinks and alike products typically contain less than 3% by weight of MSNF.

- 30 As previously mentioned, in a preferred aspect, the food product has a shelf-life of more than seven days, preferably more than 14 days, more preferably more than 28 days. In one preferred aspect the food product has a shelf-life of more than three months, preferably more than four months, preferably more than five months, such as more than six months.

## OTHER ASPECTS

In one aspect, the present invention provides a process for the production of a food product comprising the step of dissolving a stabiliser directly in a food material wherein  
5 the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

In this aspect preferably the stabiliser is in a solid form. The stabiliser may for example be in the form of a powder. The stabiliser may be in the form of a dry mix with sugar.

10

In this aspect preferably the food material comprises milk, more preferably the food material is milk.

In this aspect, preferably the process is as described herein. In this aspect, preferably  
15 the stabiliser is as described herein. In this aspect, preferably the food material is as described herein. In this aspect, preferably the process, the stabiliser and the food material are as described herein.

In one aspect the present invention provides use of a stabiliser for improving the texture  
20 and/or viscosity (such as mouthfeel and/or other organoleptic properties) of a food product, wherein the stabiliser comprises a depolymerised pectin. In this aspect preferably the stabiliser further comprises a high molecular weight, high ester pectin. In this aspect, preferably the food product is not a beverage.

25 The term "high molecular weight, high ester pectin" means a pectin having a viscosity in a 5% solution at 25°C of more than 400 cP and a degree of esterification of at least 50%.

In this aspect preferably the food product comprises the stabiliser in an amount of 0.1 to  
1 wt%, preferably 0.2 to 0.7 wt%, more preferably 0.2 to 0.5 wt%.

30

Aspects of the invention are defined in the appended claims.

The present invention will now be described in further detail in the following examples.

## **EXAMPLES**

**Objective:** To test the performance of a depolymerised pectin added to milk prior to pasteurisation, inoculation and fermentation for production of drinking yoghurt.

### **Stabilisers**

GRINDSTED™ Pectin AMD1387 was dissolved in demineralised water at 85-90 °C to constitute a 5% solution. pH was adjusted to 5.5 by addition of 20% sodium carbonate solution. The solution was kept at 80 °C for 2-8 hours until the viscosity of the solution (measured at 25 °C) was lowered to 30-50 mPa.s. Then pH was lowered to 3.5 by addition of 30% nitric acid and the mixture was cooled to room temperature. Pectin was precipitated out of the solution by pouring the mixture in 3 vol. parts 80% isopropyl alcohol under good agitation. After approx. four hours the precipitate was separated from the liquid by filtration through a cloth and washed with another part of 80% isopropyl alcohol. After pressing in the cloth the material was dried in a ventilated oven at 60 °C during the night. The dried product was milled to obtain a depolymerised pectin and the following products were prepared by similar procedure:

	DPP1	DPP2	DPP3
<b>Pectin raw material</b>	-	Citrus	-
<b>Degree of Esterification (%):</b>	70.0%	70.0%	70.0%
<b>Viscosity, 5%solution at 25°C:</b>	20 mPa.s	35 mPa.s	100 mPa.s

The depolymerised pectin having a viscosity of 35mPa.s (DPP2) was used in the following example.

The following known stabilisers were also used as comparative examples: GRINDSTED® Pectin AMD 780 (AMD 780), GRINDSTED® Pectin Wave 212 (Wave 212), and Soyafibe-S-DA 100 (soluble soybean polysaccharide, SSPS, produced by Fuji Oil Co., Ltd., Japan). GRINDSTED® products are available from Danisco A/S.

**Recipe conditions:** The final drinking yoghurt was characterised by a milk solid non-fat content (MSNF) of 8%, a sugar content of 8%, a fat content of 0.1%, and a pH of 4.0-4.1.

The stabilisers were applied in the following concentrations (% w/w of total drink composition):

DPP2	0.5%
Wave 212:	0.5%
AMD 780:	0.4%
SSPS:	0.4%
SSPS:	0.5%

- 5 **Process conditions:** Skimmed milk powder was hydrated for 30 minutes at 50°C. Stabilisers were dry mixed with 1/8 of the total sugar amount and dissolved in deionised water at 80°C. Thereafter, the stabiliser solutions were cooled to 40°C and added to recombined milk under stirring for 5 minutes. The stabiliser-milk blends were pasteurised in tank at 95°C for 10 minutes, cooled to fermentation temperature of 42°C
- 10 and inoculated with 0.02% yoghurt culture Jo-mix NM 1-20. The stabiliser-milk blends were fermented to pH 4.2 at 42°C, then they were agitated to brake down the casein curd and cooled to 10°C.

- The remaining sugar part was added to the drinking yoghurts. PH was adjusted to 4.0 by
- 15 addition of citric acid solution. At this stage the samples were divided into two parts: Homogenisation without post-pasteurisation and homogenisation combined with post-pasteurisation. Homogenisation was done at 300 bar. Samples to be pasteurised were preheated to 60°C and homogenised at 300 bar/60°C and subsequent pasteurised at 90°C for 15 seconds. All drinks were filled in bottles and stored at cold conditions.

20

- Evaluation of samples:** All samples were inspected visually 1 day after production having been stored at 5°C. In addition, all samples were inspected visually and analytically 5 days after production having been stored at 5°C. Viscosity was measured at 10°C with a Brookfield Viscometer model DVII equipped with spindle no. 6.1 and
- 25 running at 30 rpm. The reading was taken after 30 seconds. Sedimentation was accelerated by centrifugation at 2800 g for 20 min. in a Heraeus Varifuge 3.2S and expressed as the ratio of sediment to total sample. The particle size was measured in a phosphate-citrate buffer at pH 4.0 on a Malvern Mastersizer S.

### Results - 1 day post-production

#### **Non-pasteurised samples (without final pasteurisation to prolong shelf life)**

Sample	Visual inspection
DPP2, 0.5%	Fine. No separation and no sedimentation.
Wave 212, 0.5%	Weak separation and sedimentation.
AMD 780, 0.4%	Separation and sedimentation.
SSPS, 0.4%	Fine. No separation and no sedimentation.
SSPS, 0.5%	Fine. No separation and no sedimentation.
No stabiliser	Separation and sedimentation.

5

#### **Post-pasteurised samples (final pasteurisation to prolong shelf life)**

Sample	Visual inspection
DPP2, 0.5%	Fine. No separation and no sedimentation.
Wave 212, 0.5%	Weak separation and sedimentation.
AMD 780, 0.4%	Separation and sedimentation.
SSPS, 0.4%	Fine. No separation and no sedimentation.
SSPS, 0.5%	Fine. No separation and no sedimentation.
No stabiliser	Separation and sedimentation.

### Results – 5 days post-production

10

#### **Non-pasteurised samples (without final pasteurisation to prolong shelf life)**

Sample	pH	Visual inspection	Accelerated sedimentation	Viscosity	Mean diameter particle
DPP2, 0.5%	4.0	Weak separation and sedimentation	13%	6 cP	2.7 µm
Wave 212, 0.5%	n.a.	Heavy separation and sedimentation	n.a.	n.a.	n.a.
AMD 780, 0.4%	n.a.	Heavy separation and sedimentation	n.a.	n.a.	n.a.
SSPS, 0.4%	4.1	Weak separation and sedimentation	10%	3 cP	2.3 µm
SSPS, 0.5%	4.1	Weak separation and sedimentation	13%	6 cP	2.6 µm
No stabiliser	4.0	Heavy separation and sedimentation	18%	6 cP	6.0 µm

### Post-pasteurised samples (final pasteurisation to prolong shelf life)

Sample	pH	Visual inspection	Accelerated sedimentation	Viscosity	Mean particle diameter
DPP2, 0.5%	4.0	Weak separation and sedimentation	10%	6 cP	2.4 µm
Wave 212, 0.5%	n.a.	Heavy separation and sedimentation	n.a.	n.a.	n.a.
AMD 780, 0.4%	n.a.	Heavy separation and sedimentation	n.a.	n.a.	n.a.
SSPS, 0.4%	4.0	Weak separation and sedimentation	10%	2 cP	2.2 µm
SSPS, 0.5%	4.1	Weak separation and sedimentation	10%	5 cP	2.0 µm
No stabiliser	4.0	Heavy separation and sedimentation	20%	7 cP	8.8 µm

5 The samples containing Wave 212 and AMD 780 were separated totally with rather compact sedimentation. As it was impossible to re-disperse this sediment upon heavy shaking, it was not possible to characterise the drinks with these stabilisers analytically.

10 AMD 780 was included in the application trial to illustrate what generally happens when commercial pectin stabilisers are added to the application prior to fermentation. The pasteurised milk-pectin blend destabilised almost immediately and did not re-stabilise under the following processing of fermentation, homogenisation and pasteurisation.

15 Wave 212 is a HE pectin fibre product with characteristics similar to DPP2 despite the fact that it has a higher viscosity of around 242 mPa-s in a 5% solution at 25°C. AMD 780 typically has viscosity of more than 1000 mPa-s. From other test series (not reported here) we know that Wave 212 can stabilise the above drinking yoghurt recipe, when it is applied at 0.5% to the fermented yoghurt. However, the present trial indicates that the viscosity is too high for Wave 212 to be added to milk prior to fermentation without subsequent destabilisation of the milk-pectin blend.

20

SSPS is claimed to stabilise drinking yoghurt even when added to milk prior to pasteurisation, inoculation and fermentation. However, SSPS is mainly targeted at lower MSNF-contents and pH values than the applied conditions in this trial. Therefore, the

characteristics of the drinking yoghurt in the present test are not quite optimal with SSPS – sediment values of around 2-3% would be expected with the present recipe and process when stabilised with AMD 780 at normal conditions (i.e. added after fermentation to the yoghurt).

5

DPP2 demonstrates that a stabiliser comprising a depolymerised pectin can be added to milk prior to pasteurisation, inoculation, and fermentation of the milk with a stabilising performance comparable to SSPS. The milk-stabiliser blend did not separate upon pasteurisation, inoculation, and fermentation and a fairly stable product was obtained  
 10 upon homogenisation and even pasteurisation of the final drinking yoghurt. Like for SSPS, the stability of the resulting drinking yoghurt samples may not be fully optimal. Adjustment of recipe conditions (e.g. lower MSNF-content, lower pH) may improve the performance of DPP2.

15 The data illustrates that there is no detrimental effects of the second pasturisation step – and hence the invention is suitable for application in long-life products (typically of 6 month shelf life), and to food products containing live micro-organisms (typically 14 to 28 days shelf life).

## 20 VISCOSITY DETERMINATION

The viscosity was measured by the following method.

25 25.00 gram of stabiliser was dissolved in approx. 500 ml demineralised water at 80°C in a tared beaker to prepare a 5% solution.

The stabiliser solution was cooled to 25°C and pH was adjusted to  $3.5 \pm 0.2$  by addition of 1.N hydrochloric acid or 20% sodium carbonate solution.

30 The total weight of the solution was brought to 500.0 gram by dilution with demineralised water.

The viscosity was measured on a Brookfield Viscometer model DV-II with spindle No. 6.1 (Spindles No. 6.2 or 6.3 on case of higher viscosities) at 25°C at 60 rpm.)

35

SOLUTION CONCENTRATION	5%		
Stabiliser	Viscosity (mPa·s)	(Spindle No.)	pH
DPP2	35	(6.1)	3.7
Wave 212	242 -	(6.2)	3.4
SSPS	9.5 -	(6.1)	3.5
AMD780	- more than 1000	(6.3)	3.3

Wave 212, SSPS and AMD780 are comparative examples.

All publications mentioned in the above specification are herein incorporated by  
 5 reference. Various modifications and variations of the described methods and system of  
 the invention will be apparent to those skilled in the art without departing from the scope  
 and spirit of the invention. Although the invention has been described in connection with  
 specific preferred embodiments, it should be understood that the invention as claimed  
 should not be unduly limited to such specific embodiments. Indeed, various  
 10 modifications of the described modes for carrying out the invention which are obvious to  
 those skilled in chemistry, biochemistry or related fields are intended to be within the  
 scope of the following claims.



## REFERENCES

- [1] U. Einhorn-Stoll *et al.*, *Nahrung/Food*, vol. 45, pp. 332-337, 2001. Thermodynamic compatibility of sodium caseinate with different pectins. Influence of the milieu conditions and pectin modifications.
- [2] A. Marozienne *et al.*, *Food Hydrocolloids* vol. 14, pp. 391-394, 2000. Interaction of pectin and casein micelles.
- [3] Anonymous. *Food Chemical Codex* 4<sup>th</sup> edition, National Academy Press Washington DC, pp. 283-286, 1996. Pectins.
- [4] A. Syrbe *et al.*, *Int. Dairy Journal*, vol. 8, pp. 179-193, 1998. Polymer science concepts in dairy systems – an overview of milk protein and food hydrocolloid interaction.
- [5] S. H. Christensen, *FIA Conference Proceedings*, 9 pp., 1992. Protein-pectin interaction in sour milk beverages.
- [6] V. B. Tolstoguzov in *Functional properties of food macromolecules* edited by J.R. Mitchell, Elsevier Applied Science Publishers, London, pp. 385-415, 1986. *Functional Properties of Protein-Polysaccharide Mixtures*.
- [7] E. Dickinson, *Trends in Food Science & Technology*, vol. 9, pp. 347-354, 1998. Stability and rheological implications of electrostatic milk protein-polysaccharide interactions.
- [8] E. Kratz *et al.*, *Deutsche Milchwirtschaft*, vol. 32, pp. 995-996, 1992. Apfelpektine zur stabilisierung von Milchprodukten.
- [9] V.B. Tolstoguzov, *Nahrung*, vol. 42, pp. 205-209, 1998. Physico-chemical modification of food proteins: Food emulsions.
- [10] P.G. Dalev *et al.*, *Papers presented at the 3rd International Symposium of Separations for Biotechnology*, University of Reading, pp. 147-151, 1994. Use of non-specific interactions of gelating and pectin for concentration and separation of proteins and formulation of new nutritive products.
- [11] G.O. Phillips in *Emulsifiers, stabilisers and thickeners for the food industry* 1, pp.19-37, 1986. Interactions of anionic food hydrocolloids.
- [12] V. Tolstoguzov in *Macromolecular interactions in food technology* by N. Parris *et al.*, *ACS Symposium series*, pp.2-14, 1996. Structure-property relationships in foods.
- [13] L. Macfadyen, *Int. Food Ing.*, vol. ½, pp. 11-14, 1992. New uses of pectin in the dairy industry.

- [14] G. Tilly, Food Ingredients Europe: Conference Proceedings, pp. 105-121, 1991. Stabilization of dairy products by hydrocolloids.
- [15] J.M.M. Crujisen *et al.*, Netherlands Milk & Dairy Journal, vol. 48, pp. 177-180, 1994. Effect of malto-dextrins on the heat stability of caseinate emulsions.
- [16] J.-L. Doublier *et al.*, Current Opinion in Colloid & Interface Science, vol. 5, pp. 202-214, 2000. Protein-polysaccharide interactions.
- [17] R. Tuinier *et al.*, Food Hydrocolloids, vol. 14, pp. 1-7, 2000. The effect of depolymerised guar gum on the stability of skim milk.
- [18] S. Bourriot *et al.*, International Dairy Journal, vol. 9, pp. 353-357, 1999. Phase separation, rheology and structure of micellar casein-galactomannan mixtures.
- [19] H. Maeda, Handbooks of Hydrocolloids edited by G.O. Phillips and P.A. Williams, Woodhead Publishing LTD. ch. 17, pp. 309-320, 2000. Soluble soybean polysaccharide.

**CLAIMS**

1. A process for the production of a food product comprising the steps of:

(i) contacting a food material with a stabiliser to provide a food intermediate;  
and

(ii) fermenting the food intermediate;

wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

2. A process according to claim 1, further comprising, before step (ii), the step of (i)(a) pasteurising the food intermediate.

3. A process according to claim 1 or 2, further comprising, before step (ii), the step of (i)(b) inoculating the food intermediate.

4. A process according to any one of claims 1 to 3 comprising, in the following order, the steps of:

(i) contacting a food material with a stabiliser to provide a food intermediate;

(i)(a) pasteurising the food intermediate;

(i)(b) inoculating the food intermediate; and

(ii) fermenting the food intermediate.

5. A process according to any one of claims 1 to 4 further comprising the step of (iii) pasteurising the product of step (ii).

6. A process according to any one of the preceding claims further comprising the step of (iv) adding juice and/or acid to the product of step (i)(b) and/or to the product of step (ii) and/or to the product of step (iii).

7. A process according to any one of the preceding claims wherein the depolymerised pectin has a viscosity at 25°C in a 5% solution of 15mPa·s to 400mPa·s.

8. A process according to any one of the preceding claims wherein the depolymerised pectin has a viscosity at 25°C in a 5% solution of 20mPa·s to 200mPa·s.

9. A process according to any one of the preceding claims wherein the depolymerised pectin has a viscosity at 25°C in a 5% solution of 25mPa·s to 50mPa·s.
10. A process according to any one of the preceding claims wherein the depolymerised pectin is an essentially linear carbohydrate polymer.
11. A process according to any one of the preceding claims wherein the depolymerised pectin has a galacturonic acid content of at least 65%.
12. A process according to any one of the preceding claims wherein the depolymerised pectin has a degree of esterification at least 50%.
13. A process according to any one of the preceding claims wherein the depolymerised pectin has a degree of esterification of from 50 to 85%.
14. A process according to any one of the preceding claims wherein the depolymerised pectin has a degree of esterification of from 65 to 75%.
15. A process according to any one of the preceding claims wherein the protein is of animal origin and/or vegetable origin and/or microbial origin.
16. A process according to any one of the preceding claims wherein the protein is of animal origin.
17. A process according to any one of the preceding claims wherein the protein is a milk protein.
18. A process according to any one of the preceding claims wherein the food material comprises milk.
19. A process according to claim 18 wherein the milk has a milk solid non-fat content of 0.1 to 25 wt%, preferably 3 to 25 wt%, more preferably 9 to 25 wt%.
20. A process according to any one of claims 2 to 19 wherein the pasteurising step (i)(a) takes place at a temperature of at least 80°C, preferably about 95°C.

21. A process according to any one of claims 2 to 20 wherein the pasteurising step (i)(a) takes place over a period of 5 to 15 minutes, preferably about 10 minutes.
- 5 22. A process according to any one of claims 3 to 21 wherein the inoculation step (i)(b) comprises the addition of a live food-grade micro-organism.
23. A process according to claim 22 wherein the live food-grade micro-organism is a probiotic bacterium.
- 10 24. A process according to claim 22 or 23 wherein the live food grade micro-organism is selected from the list consisting of *Bifidobacteria*, *Streptococcus thermophilus*, *Lactobacilli* and mixtures thereof.
- 15 25. A process according to claim 22, 23 or 24 wherein the live food grade micro-organism is selected from the list consisting of *Bifidobacteria*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus* and mixtures thereof.
- 20 26. A process according to any one of claims 22 to 25 wherein the live food grade micro-organism comprises *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.
27. A process according to any one of the preceding claims wherein the fermentation step (ii) takes place at a temperature of from 30 to 50°C, preferably from 37 to 43°C.
- 25 28. A process according to any one of the preceding claims wherein the fermentation step (ii) takes place over a period of 2 to 48 hours.
29. A process according to any one claims 5 to 28 wherein the pasteurising step (iii) takes place at a temperature of at least 80°C, preferably about 90°C.
- 30 30. A process according to any one claims 5 to 29 wherein the pasteurising step (iii) takes place over a period of 5 to 30 seconds, preferably 10 to 20 seconds.

31. A process according to any one of the preceding claims wherein the food product is a beverage.

5 32. A process according to any one of the preceding claims wherein the food product is a fermented milk drink.

33. A process according to any one of the preceding claims wherein the food product is a yoghurt drink.

10 34. A process according to any one of the preceding claims wherein the food product is a drinking yoghurt drink.

15 35. A process according to any one of the preceding claims wherein the food product contains a live food-grade micro-organism in an amount of from 0.01 to 0.03 wt%, preferably about 0.02 wt%.

36. A process according to any one of the preceding claims wherein the food product contains the stabiliser in an amount of 0.3 to 3.0 wt%.

20 37. A process according to any one of the preceding claims wherein the food product has a pH of less than 4.6.

25 38. A process for the production of a food product comprising the step of dissolving a stabiliser directly in a food material wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

39. A process according to claim 38 wherein the stabiliser is in a solid form.

40. A process according to claim 38 or 39 wherein the food material comprises milk.

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41. A food product obtained or obtainable by the process of any one of the preceding claims.

35 42. Use of a stabiliser for improving the texture and/or viscosity of a food product, wherein the stabiliser comprises a depolymerised pectin.

43. Use according to claim 42 wherein the stabiliser further comprises a high molecular weight, high ester pectin.

**ABSTRACT****PROCESS**

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The present invention provides a process for the production of a food product comprising the steps of (i) contacting a food material with a stabiliser to provide a food intermediate; and (ii) fermenting the food intermediate; wherein the stabiliser comprises a depolymerised pectin and wherein the food material comprises a protein.

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